

## **EXHIBIT D**

From Page No. 34

Prepare hybridization mixes  
lysates:  
30  $\mu$ l DIBSS diluent  
10  $\mu$ l lysate  
360  $\mu$ l DIBSS / probe  
400  $\mu$ l

Do duplicate  
Pos. Control

30  $\mu$ l DIBSS  
1  $\mu$ l Chlamydia RNA + 9  $\mu$ l H<sub>2</sub>O ~ 10  $\mu$ l E. coli RNA  
360  $\mu$ l DIBSS / probe  
400  $\mu$ l

Incubate 1 hr 64°C

Neg. control

30  $\mu$ l DIBSS  
10  $\mu$ l 3.2% SDS  
360  $\mu$ l DIBSS / probe  
400  $\mu$ l

Add 4.5 ml Separation Solution .14M PB LN 60278

Incubate 5' at 64°C

Centrifuge

Decant SN into 30 ml scintillation vial

Add 5.0 ml Wash solution, vortex LN 60276 .14M PB

Incubate 5' at 64°C

Centrifuge

Decant SN into same scintillation vial as 1st wash

1. Count <sup>HA</sup> pellets and wash fraction for <sup>32</sup>P counts

2. Add 5.0 ml cytoscint to HA pellet  
Add 15.0 ml p gel to wash vial  
Count again to get <sup>125</sup>I counts

Used Sal for counting 2x2'

Prepare blanks  
① spin down 4.5 ml HA and count  
② add 9.0 ml .14M wash and to scint. vial and count

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Witnessed & Understood by me,  
Johann Kop

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Invented by

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Karl Forder

TITLE

Pr j ct N 3Bo k No. 405

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From Page No. 38

Purpose: To assay lysates of Chlamydia strains for cross reactivity with probes at 60°C and 176 at 64°C.

Reagents: Same as for previous experiment (405:34)

In addition used lysates for C. trachomatis 300, 301, 302, 303 (other serotypes than the one used to make the probes and found in humans.)

From Mary NB 435:3

Diluted 711 RNA 25 µg/µl (334:10) 1.5 to 1.05 µg/µl

Made up probe solutions

① To 8.4 ml DIBS3 mix, add 5 µl of probe 176 ( $1.3 \times 10^4$  cpm/µl) NB 391:25 kinase and 20 µl of probe 1082 (22,000 cpm/µl) iodinated by Kevin NB 388:42

②

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Witnessed &amp; Understood by me,

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TITLE Specificity Studies with *Chlamydia* probes

Pr j ct N 3  
Bo k N 405

40

From Page No.     

Assay Protocol: Prepare hybridization mixtures in scintillation vials:

Lysates: 30  $\mu$ l DIBSS diluent  
10  $\mu$ l lysate  
360  $\mu$ l DIBSS/probe mix  
400  $\mu$ l total volume

N.C.: 30  $\mu$ l DIBSS diluent  
10  $\mu$ l 3.2% SDS  
360  $\mu$ l DIBSS/probe mix  
400

711 RNA: 30  $\mu$ l DIBSS diluent  
2  $\mu$ l RNA 105  $\mu$ g/ml  
8  $\mu$ l H<sub>2</sub>O  
360  $\mu$ l DIBSS/probe  
400

E. coli RNA: 30  $\mu$ l DIBSS diluent  
10  $\mu$ l RNA 105  $\mu$ g/ml  
360  $\mu$ l DIBSS/probe  
400

Incubate ChtA 176 probe rxn at 64°C 1 hr.

Separate hybrids and wash as <sup>405</sup>p. 35

Count in HAL 2x2' on  
to get <sup>32</sup>P counts Program 1

Add 0.09 ml of probe mixture to HA pellet in scint vial  
Add 5.0 ml cytosol and count use this value  
for total counts for <sup>25</sup>I-hybridization w/ pan bacterial probe  
Add 5.0 ml cytosol, and count in HAL 2x2'  
to get <sup>125</sup>I counts. ~~etc~~ Program 1 <sup>10845134</sup>  
(Did not count weaker for <sup>125</sup>I as did in previous expt)

E. coli RNA sample 2 was spilled for probe ChtA 176.

Results: see p. 41

Conclusions: Probes 176 and reacted with all *C. trachomatis*  
strains but not with the *C. psittaci*. The <sup>125</sup>I-<sup>1082</sup>  
data showed that RNA was present in all tubes

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Witnessed & Understood by me,

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TITLE Specificity Study of Chlamydia probes

Proj ct N 3  
B k N 405

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From Page No. 40

		CA/A 176 E/A 102	320		
	N	SAMPL	CPM	TOTAL	%
711	1	FP	929.8	2091.0	44.5
		BP	1161.3		55.5
	2	FP	942.3	2166.0	43.5
		BP	1223.8		56.5
300	3	FP	623.0	2210.0	28.2
		BP	1587.0		71.8
	4	FP	631.8	2179.3	29.0
		BP	1547.5		71.0
301	5	FP	561.5	2180.3	25.8
		BP	1618.8		74.2
	6	FP	536.8	2159.5	24.9
		BP	1622.8		75.1
302	7	FP	566.5	2147.5	26.4
		BP	1581.0		73.6
	8	FP	610.8	2248.3	27.2
		BP	1637.5		72.8
303	9	FP	551.3	2178.0	25.3
		BP	1626.8		74.7
	10	FP	563.5	2124.0	26.5
		BP	1560.5		73.5
794	23	FP	2278.5	2295.0	99.3
		BP	16.5		.7
	12	FP	2313.5	2328.0	99.4
		BP	14.5		.6
767	13	FP	2228.5	2239.3	99.5
		BP	10.8		.5
	14	FP	2284.0	2295.5	99.5
		BP	11.5		.5
768	15	FP	2303.3	2319.5	99.3
		BP	16.3		.7
	16	FP	2302.5	2318.3	99.3
		BP	15.8		.7
711 RNA	17	FP	403.0	2152.0	18.7
		BP	1749.0		81.3
	18	FP	310.0	2073.8	14.9
		BP	1763.8		85.1
ali	19	FP	2202.8	2217.3	99.3
		BP	14.5		.7
	20	FP	590.8	597.3	98.9
		BP	6.5		1.1
NC	21	FP	2290.5	2316.3	98.9
		BP	25.8		1.1
	22	FP	2255.3	2278.0	99.0
		BP	22.8		1.0

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From Page No. \_\_\_\_\_

Results:

EcoR 1082 with 176

TOTAL 1: 13760

SAMPLE	CPM	HAZ	AVGZ	XBKGD
711	6849	49.8		
	7170	52.1	50.9	
300	8420	61.2		
	8571	62.3	61.7	
301	9490	69.0		
	9610	69.8	69.4	
302	8809	64.0		
	9557	69.5	66.7	
303	9153	66.5		
	8793	63.9	65.2	
754	7520	54.7		
	7666	55.7	55.2	
767	7692	55.9		
	7477	54.3	55.1	
768	4677	34.0		
	4483	32.6	33.3	
RNA 711	8877	64.5		
	9516	69.2	66.8	
coli	8431	61.3	61.3	
N.C.	139	1.0	1.0	
	132	1.0	1.0	

Lysates		<sup>32</sup> P-CntA 176	<sup>32</sup> P-EcoA 1082
C. trachomatis	711	56.0	50.9
"	300	71.4	61.7
"	301	74.5	69.4
"	302	73.2	66.7
"	303	74.1	65.2
C. psittaci	754	0.7	55.2
"	767	0.5	55.1
"	768	0.7	33.3
RNA			
C. trachomatis	711	83.2	66.8
E. coli		0.9	61.3
N.C.		1.1	1.0

Hybridization  
Assays done at 60°C

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